



Strong nuclear differentiation contrasts with widespread sharing of plastid DNA haplotypes across taxa in European purple saxifrages (*Saxifraga* section *Porphyron* subsection *Oppositifoliae*)

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The purple saxifrages, *Saxifraga* section *Porphyron* subsection *Oppositifoliae*, comprise the closest relatives of the arctic–alpine model plant *S. oppositifolia* and have a centre of diversity in the central and southern European mountain ranges. Many taxa have been described and taxonomic concepts vary among different treatments. Using amplified fragment length polymorphism (AFLP) fingerprinting, we show that some taxa form strongly supported genetic entities best recognized at the species level (*S. biflora*, *S. blepharophylla*, *S. retusa*, *S. rudolphiana* and *S. speciosa*), whereas others (*S. murithiana* and *S. paradoxa*) are not genetically divergent at all. *Saxifraga oppositifolia* s.s. is phylogenetically incoherent. Plastid DNA sequence data show limited congruence with the predominantly nuclear-derived AFLPs. Several co-distributed taxa (*S. biflora*, *S. blepharophylla*, *S. oppositifolia* s.s. and *S. retusa*) share the same set of haplotypes. In the widespread *S. oppositifolia* and *S. retusa*, highly divergent haplotype lineages were discovered which exhibit a geographical rather than taxonomic structure. Recent and ancient hybridization and/or lineage sorting are probably responsible for the strong incongruence between data derived from nuclear and plastid genomes. Hybridization, which is known to occur among almost all taxa of this group when growing in sympatry, however, seems to be insufficient to break down species barriers. © 2013 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2013, 173, 622–636.

ADDITIONAL KEYWORDS: AFLP – chloroplast capture – hybridization – plastid capture – phylogenetic analysis – *Saxifraga oppositifolia* – taxonomy.

INTRODUCTION

Saxifraga oppositifolia L. (Saxifragaceae) has become a model system for the evolution and spatiotemporal diversification of arctic–alpine plants (Holderegger &

Abbott, 2003). Focusing on the circumpolar range, a clear separation of two clades with amphi-Atlantic and amphi-Beringian distribution was manifested on the basis of restriction fragment length polymorphisms (RFLPs; Abbott *et al.*, 2000; Abbott & Comes, 2004) and plastid DNA sequences (Holderegger & Abbott, 2003). These two groups were confirmed by an extended sample of plastid DNA sequences and by

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Table 1. European taxa of *Saxifraga* section *Porphyron* subsection *Oppositifoliae* included in the present study. Previous taxonomy and distribution mostly follow Webb & Gornall (1989) and Webb (1993); exceptions are stated in the ‘Study species’ section. Habitat requirements and distribution follow Grabherr & Mucina (1993), Hayek (1905), Schönswetter *et al.* (2000), Webb & Gornall (1989) and the authors’ field observations

Previous taxonomy	Taxonomy suggested by the present study	Habitat	Distribution
<i>S. biflora</i> All. ssp. <i>biflora</i>	<i>S. biflora</i> All.	Upper alpine to nival, mainly calciferous schist, rarely limestone, unconsolidated scree	Alps
<i>S. oppositifolia</i> L.			
Ssp. <i>blepharophylla</i> (Kern. ex Hayek) Vollm.	<i>S. blepharophylla</i> Kern. ex Hayek	Upper alpine to subnival, basic silicates and marble, rocky outcrops	Eastern Alps
Ssp. <i>oppositifolia</i> (including <i>S. meridionalis</i> Terr. and <i>S. murithiana</i> Tiss.)	<i>S. oppositifolia</i> L. ssp. <i>oppositifolia</i>	(Montane) subalpine to nival, mainly calciferous bedrock (especially limestone and calciferous schists), more rarely siliceous bedrock, consolidated scree and rocks	Arctic–alpine, circumpolar
Ssp. <i>paradoxa</i> D.A.Webb	<i>S. oppositifolia</i> L. ssp. <i>oppositifolia</i>	(Subalpine) alpine to nival, mainly calciferous, more rarely siliceous bedrock, consolidated scree and rocks	Pyrenees
Ssp. <i>rudolphiana</i> (Hornsch. ex Koch) Nym.	<i>S. rudolphiana</i> Hornsch. ex Koch	Upper alpine to nival, basic bedrock (marble or calciferous schist), open, snow-rich communities and rocks	Eastern Alps, probably Carpathians
Ssp. <i>speciosa</i> (Dörf. & Hayek) Engl. & Irmsch.	<i>S. speciosa</i> Dörf. & Hayek	Alpine limestone rocks and scree	Central Apennines (Abruzzo)
<i>S. retusa</i> Gouan	<i>S. retusa</i> Gouan		
Ssp. <i>augustana</i> (Vacc.) P.Fournier (= <i>S. purpurea</i> All.)	<i>S. retusa</i> Gouan ssp. <i>augustana</i> (Vacc.) P.Fournier	Alpine, mostly calciferous schists, exposed and windswept habitats	Western Alps
Ssp. <i>retusa</i>	<i>S. retusa</i> Gouan ssp. <i>retusa</i>	Mainly upper alpine, siliceous bedrock, exposed, windswept, consolidated scree and rocks	Pyrenees, Alps, Carpathians, Rila (Bulgaria)

amplified fragment length polymorphisms (AFLPs; Winkler *et al.*, 2012). However, the inclusion of southern and south-eastern European and central Asian populations showed that the amphi-Beringian clade probably originated in central Asia, but extends westwards as far as the Apennines and Carpathians, where it meets the amphi-Atlantic clade. This pattern, with a possible origin of the amphi-Atlantic clade in the Alps (Winkler *et al.*, 2012), emphasizes the important role of central and southern European mountain ranges (i.e. the Pyrenees, Apennines, Alps, Carpathians and mountains of the Balkan Peninsula) in the evolution of *S. oppositifolia* in particular and of arctic–alpine species in general (Schönswetter *et al.*, 2003; Ronikier, 2011; Ronikier, Schneeweiss & Schönswetter, 2012).

Our good understanding of the phylogeographical history of *S. oppositifolia* contrasts with our limited knowledge of the precise circumscription of this species concerning both the delimitation of intraspecific taxa and the phylogenetic relationships to other purple saxifrages. Although this species group, traditionally classified as section *Porphyron* subsection *Oppositifoliae* (Hayek, 1905; Engler & Irmscher, 1916–1919), is morphologically well defined by pink, purple or, rarely, white petals and opposite leaf arrangement (Webb & Gornall, 1989; Table 1), the taxonomy of *S. oppositifolia* s.l. is convoluted and poorly resolved. Apart from the widespread *S. oppositifolia* ssp. *oppositifolia*, morphologically only weakly differentiated and/or poorly known entities of uncertain taxonomic value have been described

from the amphi-Beringian Arctic [*S. oppositifolia* ssp. *glandulispala* Hultén, *S. oppositifolia* ssp. *smalliana* (Engl. & Irmsch.) Hultén; Aiken, LeBlanc & Boles, 2005], central Asian mountain ranges (*S. ajanica* Sipliv., *S. asiatica* Hayek; e.g. Hayek, 1905), south-eastern Europe (*S. meridionalis* Terr.; Hayek, 1905) and the Alps (*S. murithiana* Tiss.; Webb & Gornall, 1989). The central and southern European mountain ranges host further subspecies of *S. oppositifolia*, which are morphologically and partly also ecologically differentiated and are thus more widely accepted. These are *S. oppositifolia* ssp. *paradoxa* D.A. Webb from the Pyrenees, ssp. *speciosa* (Dörfl. & Hayek) Engl. & Irmsch. from the central Apennines, ssp. *rudolphiana* (Hornsch. ex Koch) Nym. from the eastern Alps and probably the Carpathians (see 'Study species' section) and ssp. *blepharophylla* (Kern. ex Hayek) Vollm. from the eastern Alps (Webb & Gornall, 1989; Table 1). The central and southern European mountain ranges additionally host two morphologically distinct species (Table 1): *Saxifraga biflora* All. is widespread in the Alps, whereas *S. retusa* Gouan is widely but disjunctly distributed in the Pyrenees, Alps, Carpathians and Bulgarian Rila Mountains. In *S. retusa*, the type subspecies spans the entire range of the species, whereas ssp. *augustana* (Vacc.) P. Fournier (synonym *S. purpurea* All.) is endemic to the south-western Alps, where it co-occurs with ssp. *retusa*. In the Alps, morphological delimitation is further complicated by frequent hybridization between sympatric taxa (e.g. between *S. oppositifolia* ssp. *oppositifolia* and *S. biflora*: Engler & Irmscher, 1916–1919; Hörandl & Gutermann, 1994).

The only available molecular phylogenetic study of *S. oppositifolia* and related taxa is based on nuclear ribosomal internal transcribed spacer (ITS) and plastid DNA sequences (Holderegger & Abbott, 2003). Whereas ITS sequences failed to establish well-defined entities because of insufficient resolution, plastid DNA data revealed a geographical rather than a taxonomic structure, which was interpreted as the result of incomplete lineage sorting. Because of limited sampling outside *S. oppositifolia* ssp. *oppositifolia* (only one sample each of *S. oppositifolia* ssp. *smalliana*, ssp. *blepharophylla* and *S. biflora* was included), Holderegger & Abbott (2003) were not able to draw any taxonomic conclusions, rendering our understanding of the phylogenetic relationships in this taxonomically intricate group marginal.

Here, we have investigated the phylogenetic relationships of *S. oppositifolia* and relatives, focusing on the centre of taxonomic diversity in the Alps and other central and southern European mountain ranges. To this end, we obtained data from predominantly nuclear-derived (Bussell, Waycott & Chappill, 2005) and highly resolving AFLP markers and from

maternally inherited (in Saxifragaceae: Soltis, Soltis & Ness, 1990) plastid DNA sequences using a comprehensive sampling of nearly all currently recognized central and southern European representatives of *Saxifraga* section *Porphyron* subsection *Oppositifoliae*. In order to embed our results in a geographically wider context, we complemented the newly generated data with previously published data of *S. oppositifolia* s.l. covering the Arctic, Scandinavia, the Urals and central Asia (Winkler *et al.*, 2012). We tested: (1) whether it is possible to corroborate genetically the distinctness of geographically restricted subspecies of *S. oppositifolia* (eastern Alpine *S. oppositifolia* ssp. *blepharophylla*, eastern Alpine and probably Carpathian *S. oppositifolia* ssp. *rudolphiana*, Pyrenean *S. oppositifolia* ssp. *paradoxa*, Apennine *S. oppositifolia* ssp. *speciosa*) and *S. retusa* (south-western Alpine *S. retusa* ssp. *augustana*) from the more widespread nominate taxa *S. oppositifolia* ssp. *oppositifolia* and *S. retusa* ssp. *retusa*, as suggested by morphology; (2) whether gene flow between Alpine taxa, as evidenced by the presence of hybrids, especially between *S. oppositifolia* ssp. *oppositifolia* and *S. biflora* (Hörandl & Gutermann, 1994; Gugerli, 1997), is sufficiently frequent to blur species boundaries; and (3) whether there is evidence for geographical sorting of plastid lineages, as seen in *S. oppositifolia* ssp. *oppositifolia* (Holderegger & Abbott, 2003), in other taxa, especially *S. retusa* ssp. *retusa*, the distribution of which spans the deep genetic split of *S. oppositifolia* between the Alps and the Carpathians (Winkler *et al.*, 2012). Based on our results, we suggest an improved taxonomic concept for the European taxa of *Saxifraga* section *Porphyron* subsection *Oppositifoliae*.

MATERIAL AND METHODS

STUDY SPECIES

We follow the taxonomic concept of Webb & Gornall (1989) for the European taxa, consequently subsuming populations pertaining to *S. (oppositifolia* ssp.) *murithiana* and *S. (oppositifolia* ssp.) *meridionalis* under *S. oppositifolia* ssp. *oppositifolia*. Our taxon sampling includes most European members of *Saxifraga* section *Porphyron* subsection *Oppositifoliae* (Table 1). One exception is *S. biflora* ssp. *epirotica* D.A. Webb (endemic to the Tymfi mountains in northern Greece), which is almost certainly a low-elevation form of *S. oppositifolia* and was collected only once (Authier, 1997). From *S. oppositifolia* ssp. *rudolphiana*, we only included accessions from the Alps; reports from the Romanian Carpathians (e.g. Webb, 1993; Ciocârlan, 2000), which are documented by a correctly determined herbarium specimen stored in

WU (Bucegi, Transilvania, Herb. Baumg.; missing data on label: collector, date of collection), are probably incorrect (possibly a mix-up of herbarium labels; E. Hörandl, University of Göttingen, Germany, pers. comm.). They could not be confirmed during independent field trips by E. Hörandl, P. Schönswetter and M. Puşcaş (Babeş-Bolyai University, Cluj, Romania) or by checking the herbaria BUCA, BVS, CL, CRAI, IAGB, IASI, SIB, W and WU, which contained no specimens determined as *S. (oppositifolia ssp.) rudolphiana* from the Carpathians or only incorrectly determined specimens. The presence of *S. oppositifolia ssp. rudolphiana* in the Carpathians was already doubted by Hayek (1905).

Hybridization, which is known to involve almost all Alpine taxa of the purple saxifrages (Hayek, 1905), is particularly frequent between *S. biflora* and *S. oppositifolia ssp. oppositifolia* (Hörandl & Gutermann, 1994; Gugerli, 2000). The resulting morphological intermediates (*S. × kochii* Hornung), which have often been interpreted as separate subspecies of *S. biflora* [*S. biflora ssp. macropetala* (Kern. ex Engl.) Rouy & Camus], are cross-compatible with the parental taxa (Hörandl & Gutermann, 1994; Gugerli, 2000) and can occur, despite reduced seed set, as (half-) orphans in self-sustaining populations (Gugerli, 1997). In the present study, we did not include morphologically identifiable hybrid accessions.

PLANT MATERIAL, DNA ISOLATION, PLASTID DNA SEQUENCING AND AFLP FINGERPRINTING

Leaf material of one to three individuals per sampling site was collected and immediately stored in silica gel (Table A1, including voucher numbers). Total genomic DNA was extracted from 10 mg of dried tissue with the DNeasy 96 plant mini kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The *psbA-trnH* intergenic spacer of plastid DNA was amplified and sequenced as described in Holderegger & Abbott (2003). The plastid *trnT_{UGU}-trnL_{UAA}-trnF_{GAA}* intergenic spacers including the *trnL_{UAA}* intron (hereafter referred to as *trnT-F*) were amplified, purified and sequenced as described in Winkler *et al.* (2012).

AFLPs have been widely used in phylogenetic reconstruction in recent years, particularly in groups of closely related organisms (reviewed in Meudt & Clarke, 2007), where the phylogenetic accuracy of AFLPs is only marginally compromised by a lack of band homology (García-Pereira, Caballero & Quesada, 2010). AFLP data were generated following the procedure of Vos *et al.* (1995) with the modifications described by Schönswetter *et al.* (2009). To test the reproducibility of AFLP fragments and to allow for an estimation of the error rate, 13 samples were replicated starting from the restriction/ligation step.

As in our previous study on *S. oppositifolia s.s.* (Winkler *et al.*, 2012), the three primer combinations for selective polymerase chain reaction (PCR) (fluorescent dyes in parentheses) were *EcoRI* (6-FAM)-ACA/*MseI*-CAC, *EcoRI* (VIC)-AGG/*MseI*-CTC, *EcoRI* (NED)-ACC/*MseI*-CAG. The selective PCR products were purified and subjected to electrophoresis as described in Schönswetter *et al.* (2009).

DATA ANALYSES

For plastid DNA data, a statistical parsimony network was constructed from the concatenated sequence data using TCS 1.21 (Clement, Posada & Crandall, 2000), treating sequence gaps as fifth character states after reducing insertions/deletions (indels) longer than 1 bp to single base pair indels, excluding polymorphic mononucleotide repeats and setting the connection limit to 95%. Maximum parsimony (MP) analyses and MP bootstrap analyses were performed using PAUP 4.0b10 (Swofford, 2002). The most-parsimonious trees were searched heuristically with 1000 replicates of random sequence addition, tree bisection–reconnection (TBR) branch swapping and MulTrees on. The swapping was performed on a maximum of 1000 trees (nchuck = 1000). All characters were equally weighted and unordered. The dataset was bootstrapped using 2000 replicates, TBR branch swapping, MulTrees off and random sequence addition with five replicates.

AFLP data were aligned with GeneScan 500 ROX internal size standard (Applied Biosystems, Foster City, CA, USA) and manually scored using DAX (Van Mierlo Software Consultancy, Eindhoven, the Netherlands) as described in Bendiksbj *et al.* (2011). The AFLP error rate was calculated as the number of mismatches (i.e. 0/1 or 1/0) divided by the number of matches (i.e. 0/0 and 1/1) in each pair of replicates (Bonin *et al.*, 2004). Fragments with mismatches in more than one replicate pair were omitted from analysis. Using SPLITSTREE 4.8 (Huson & Bryant, 2006), a NeighborNet diagram was produced from Nei–Li distances (Nei & Li, 1979). Node support was estimated in a neighbor-joining analysis based on Nei–Li distances and 1000 bootstrap pseudo-replicates.

RESULTS

PLASTID DNA

Sequences of the *psbA-trnH* intergenic spacer were 197–228 bp in length, and those of the *trnT-F* region were 1209–1619 bp in length. The combined alignment was 1886 bp in length (228 bp from the *psbA-trnH* intergenic spacer and 1658 bp from the *trnT-F* region) and comprised 53 variable characters, 48 of

which were parsimony informative; 34 were nucleotide substitutions and 19 were indels (2.81% variability). The exclusion of nine polymorphic mononucleotide repeats gave a total of 32 haplotypes in 140 individuals analysed. The original alignment is available on DRYAD (<http://doi.org/10.5061/dryad.8d820>). GenBank accession numbers are provided in Table A1.

In the statistical parsimony network (Fig. 1A; tree length, 72; consistency index, 0.972; retention index, 0.998), haplotype h32, found in all accessions of both subspecies of *S. retusa* from the western Alps south of the Aosta valley, could not be connected with the other haplotypes at a connection limit of 95%. All other haplotypes fell into two main lineages, previously termed Europe-centred Clade (EC-Clade) and Asia-centred Clade (AC-Clade; Winkler *et al.*, 2012). Both the EC-Clade and AC-Clade received high support in the MP analysis [bootstrap support (BS) \geq 98]. The EC-Clade was distributed in the northern Iberian Peninsula, the Alps, the western Carpathians, the Atlantic Arctic and eastern Canada, and its haplotypes were found in *S. biflora*, *S. oppositifolia* ssp. *blepharophylla*, *S. oppositifolia* ssp. *oppositifolia* from the Cordillera Cantabrica, the Alps, the western Carpathians, the Atlantic Arctic and eastern Canada, *S. oppositifolia* ssp. *paradoxa* and two populations of *S. retusa* ssp. *retusa* from the western Alps. The AC-Clade occurred in the Apennines, the eastern and southern Carpathians, mountains of the Balkan Peninsula, central and northern Asia and Beringia, and its haplotypes were found in *S. oppositifolia* ssp. *rudolphiana*, *S. oppositifolia* ssp. *speciosa* and *S. oppositifolia* ssp. *oppositifolia* from the Carpathians, the Balkan Peninsula, central and northern Asia, Beringia and northern Greenland, and in *S. retusa* ssp. *retusa* from the eastern Alps and the Carpathians.

AFLP

The error rate was 1.6%, and 18 non-reproducible fragments were removed from the dataset. A total of 575 reproducible AFLP bands was scored for 140 individuals. Eight bands found in all or all but one individual were excluded (no bands occurring in a single individual only were found); thus, further analyses were based on 567 variable AFLP bands.

The NeighborNet diagram with BS derived from neighbor-joining analysis resolved several strongly supported entities (Fig. 2). These included *S. biflora*, *S. oppositifolia* ssp. *blepharophylla*, *S. oppositifolia* ssp. *rudolphiana* and *S. oppositifolia* ssp. *speciosa*, each strongly supported with 100% BS. *Saxifraga retusa* s.l. was similarly divergent (97% BS) and fell into two main groups. One was highly supported (97% BS) and comprised populations of ssp. *retusa* from the

eastern Alps and the Carpathians. The second, unsupported group consisted of accessions of both ssp. *augustana* and ssp. *retusa* from the western Alps. *Saxifraga oppositifolia* ssp. *oppositifolia* fell into several, mostly unsupported regional groups. The first group comprised accessions from the Alps and the northern Iberian Peninsula (including ssp. *paradoxa*) and northern accessions from Newfoundland and north-western Greenland to the northern Urals and the Taymyr Peninsula. The second group comprised samples from the Balkans and the Carpathians (74% BS). The third group included samples from central and northern Asia (< 50% BS).

DISCUSSION

The purple saxifrages (*Saxifraga* section *Porphyrium* subsection *Oppositifoliae*), containing the arctic-alpine model species *S. oppositifolia* (Abbott & Comes, 2004), have their centre of diversity in southern and central Europe. Distinction between and relationships among taxa have been controversial, with taxonomic concepts varying strongly across different treatments (e.g. Pignatti, 1982; Aeschmann *et al.*, 2004; Fischer, Oswald & Adler, 2008). Consistent morphological differentiation among the widespread *S. oppositifolia* ssp. *oppositifolia* and the central European endemics *S. oppositifolia* ssp. *blepharophylla*, *S. oppositifolia* ssp. *rudolphiana* and *S. biflora* (Hörandl & Gutermann, 1994) contrasts with frequent hybridization (Gugerli, 1997) and the lack of genetic differentiation revealed by ITS and plastid DNA sequence data (Holderegger & Abbott, 2003). Conventionally used sequence markers may, however, be insufficiently variable to resolve phylogenetic relationships among closely related plant species, which can be alleviated by the use of highly polymorphic AFLPs (Després *et al.*, 2003), an approach we follow here.

Our AFLP data corroborate the distinctness of several morphologically and ecologically differentiated entities within *S. oppositifolia* corresponding to *S. oppositifolia* ssp. *blepharophylla*, *S. oppositifolia* ssp. *rudolphiana* and *S. oppositifolia* ssp. *speciosa* (Fig. 2). In contrast, other accessions of *S. oppositifolia* fall into several mostly unsupported, but geographically structured, gene pools: (1) the Alps and the northern Iberian Peninsula pertaining to *S. oppositifolia* ssp. *oppositifolia* (including *S. murithiana*) and to *S. oppositifolia* ssp. *paradoxa*, plus the North Atlantic region from Newfoundland to the Taymyr Peninsula in northern Siberia corresponding to *S. oppositifolia* ssp. *oppositifolia*; (2) the Balkans and the Carpathians pertaining to *S. oppositifolia* ssp. *oppositifolia* (including *S. meridionalis*); (3) central and northern Asia corresponding to *S. oppositifolia* ssp. *oppositifolia*

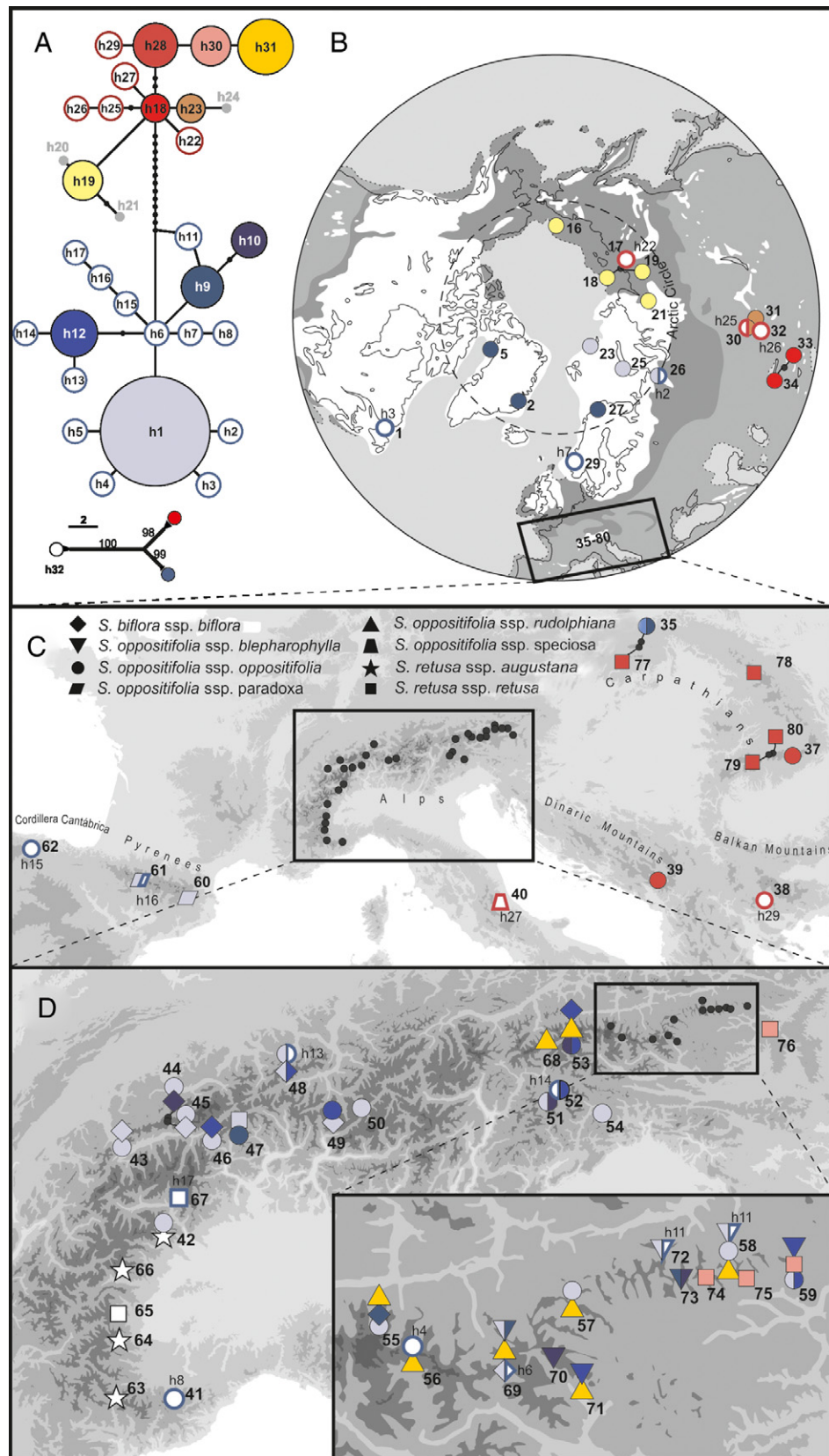


Figure 1. See caption on next page.

Figure 1. Sample sites (numbered 1–80; for details, see Table A1) and patterns of plastid DNA (*psbA-trnH*, *trnT-F*) variation in European purple saxifrages (*Saxifraga* section *Porphyrium* subsection *Oppositifoliae*). A, Statistical parsimony network of plastid DNA haplotypes. Haplotype h32 sampled in all populations of *S. retusa* s.l. in the western Alps south of the Aosta valley could not be joined with the remaining haplotypes h1–h31 at a connection limit of 95%. Small black dots represent unsampled haplotypes. Grey dots represent haplotypes sampled in a circumpolar study of *S. oppositifolia* s.s. (Winkler *et al.*, 2012) for which no amplified fragment length polymorphism (AFLP) data were available. The tree is a simplified, unrooted and bootstrapped (2000 replicates) maximum-parsimony analysis depicting the relationships among the three main haplotype groups. B–D, Distribution of sampling sites and plastid haplotypes in the Northern Hemisphere (B), southern and central Europe (C) and European Alps (D). Colour coding of haplotypes sampled at least three times corresponds to (A). Haplotypes that were sampled only once or twice are given with white filling and an indication of the respective haplotypes. The distributions of ice cover (white) and tundra (dark grey) at the last glacial maximum (for the Kara ice sheet, the maximum glacial extent is given) in (B) are modified from Frenzel (1968), Frenzel, Pécsi & Velichko (1992) and Ehlers, Gibbard & Hughes (2011). Margins of exposed continental shelves at the last glacial maximum are indicated by broken lines.

(probably including *S. asiatica*, see below). Although AFLP data confirm the distinctness of the taxonomically widely acknowledged species *S. biflora* and *S. retusa*, they fail to support the separation of *S. retusa* ssp. *retusa* from *S. retusa* ssp. *augustana*, because accessions of *S. retusa* ssp. *retusa* from the western Alps group phylogenetically with the geographically close but morphologically distinct *S. retusa* ssp. *augustana* rather than with *S. retusa* ssp. *retusa* from the eastern Alps and the Carpathians (Fig. 2).

Although based on a relatively limited number of characters, plastid DNA sequences revealed a markedly different diversification pattern than AFLPs, in that main haplotype groups were structured geographically rather than taxonomically. This is the case for *S. oppositifolia* s.l., in which two strongly divergent plastid DNA clades, the North Atlantic and European EC-Clade (containing *S. oppositifolia* ssp. *oppositifolia* including *S. murithiana*; *S. oppositifolia* ssp. *paradoxa*; *S. oppositifolia* ssp. *blepharophylla*) and the south-eastern European to Asian and North American AC-Clade (containing *S. oppositifolia* ssp. *oppositifolia*, including *S. oppositifolia* ssp. *meridionalis*; *S. oppositifolia* ssp. *rudolphiana*; *S. oppositifolia* ssp. *speciosa*; Fig. 1) have been recognized previously (Winkler *et al.*, 2012), albeit based on a less comprehensive taxonomic sampling. The discrepancy between genetically defined lineages and taxonomically delimited groups is even more pronounced in *S. retusa* s.l., the morphologically most distinct taxon of subsection *Oppositifoliae* (Hayek, 1905). This species harboured haplotypes from all three main plastid clades. (1) A haplotype (h32) that falls clearly outside the hitherto known plastid variation in *S. oppositifolia* (Winkler *et al.*, 2012) and remains unconnected in the parsimony network (Fig. 1A) was found in *S. retusa* ssp. *augustana* and *S. retusa* ssp. *retusa* from the south-western Alps south of the Valle d'Aosta, an important biogeographical border in the Alps (Schönswetter *et al.*, 2005; Thiel-Egenter *et al.*,

2011). (2) Populations of *S. retusa* ssp. *retusa* from the western Alps north and east of the Valle d'Aosta contained haplotypes of the EC-Clade, which may (h1) or may not (h17) be shared with other species (*S. biflora* and *S. oppositifolia* s.l.; Fig. 1D). (3) Populations from the eastern Alps and the Carpathians possessed haplotypes belonging to the AC-Clade, which may (h28 in the Carpathians) or may not (h30 in the Alps) be shared with other species (*S. oppositifolia* s.l.; Fig. 1C, D). Consequently, the deep genetic break between Alpine and Carpathian haplotypes seen in *S. oppositifolia* ssp. *oppositifolia* (Winkler *et al.*, 2012) recurs in *S. retusa* ssp. *retusa*, although shifted towards the west to be situated within the Alps. This genetic pattern is reflected in the distributions of several alpine species spanning the easternmost Alps and (parts of) the Carpathians. Prominent examples are *Anthemis carpatica* Waldst. & Kit. ex Willd., *Gentiana frigid* Haenke, *Ranunculus crenatus* Waldst. & Kit. and *Saxifraga carpatica* Sternb. (Schneeweiss & Schönswetter, 1999). Finally, haplotypes found in *S. biflora* belonged to the EC-Clade and, with the exception of a single haplotype (h6), were all shared with *S. oppositifolia* ssp. *oppositifolia* (h1, h9, h10, h12; Fig. 1C, D). With respect to the geographical distributions of the two clades identified previously in *S. oppositifolia* (Winkler *et al.*, 2012), the inclusion of close relatives of *S. oppositifolia* leaves the overall distribution of the EC-Clade unaffected, but extends that of the AC-Clade into the eastern Alps.

One factor probably contributing to the high level of incongruence between nuclear and plastid data is hybridization. For instance, hybrids between *S. oppositifolia* ssp. *oppositifolia* and *S. biflora* (*S.* × *kochii*) are widespread in the Alps and occasionally even occur as (half-)orphans (Hörandl & Gutermann, 1994), contributing to the former recognition of some hybrid forms as a distinct taxon (*S. biflora* ssp. *macropetala*). At the western edge of the distribution of *S. oppositifolia* ssp. *blepharophylla*

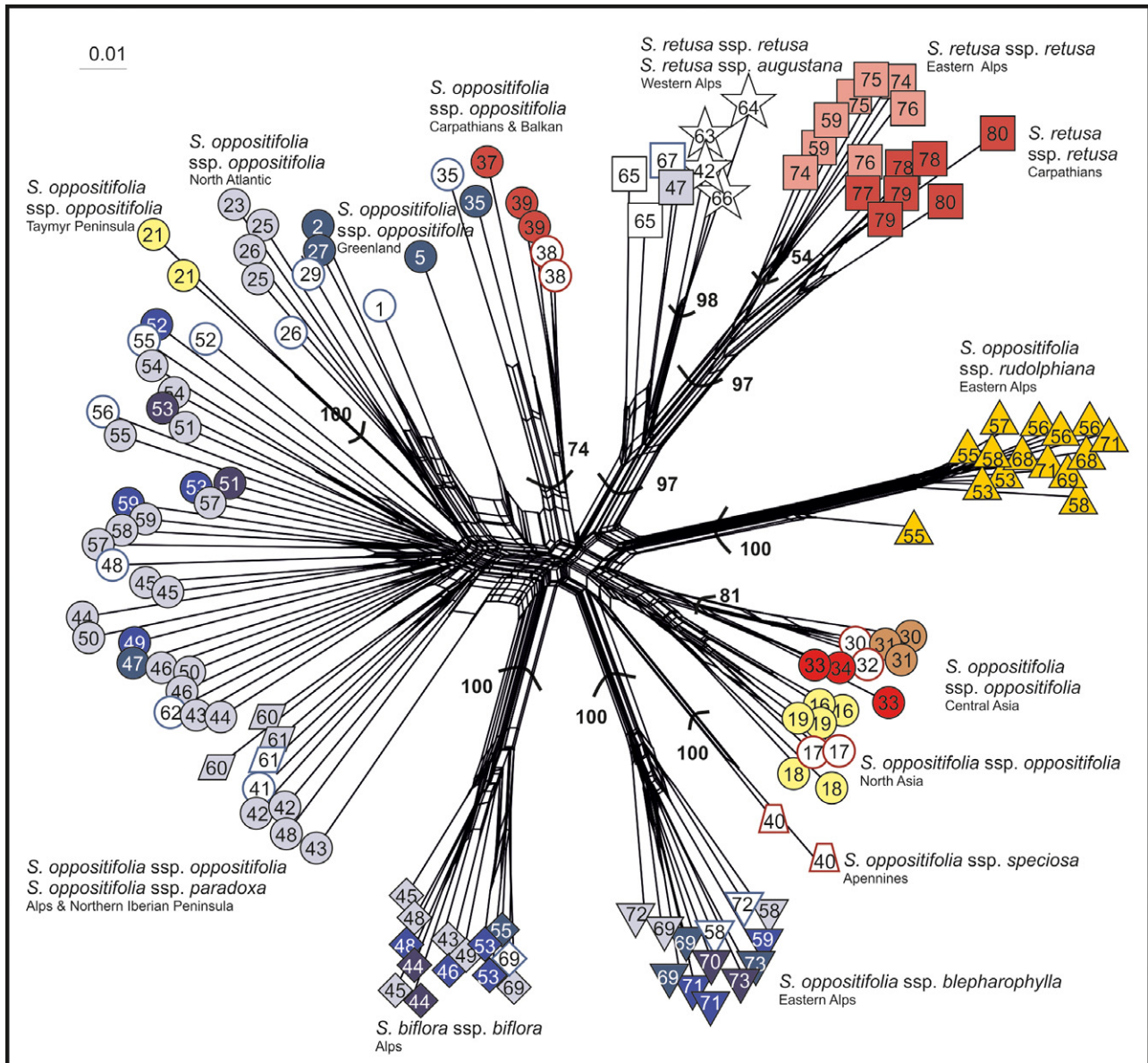


Figure 2. NeighborNet diagram of amplified fragment length polymorphism (AFLP) data sampled from European purple saxifrages (*Saxifraga* section *Porphyron* subsection *Oppositifoliae*). Numbers along the splits are the neighbor-joining bootstrap values > 50% given for the major branches only. Population numbers, taxa and plastid DNA haplotypes (Table 1) are given for each individual at the tips of the splits with colours, symbols and numbers as given in Figure 1.

in the Ankogelgruppe of the Austrian Hohe Tauern, morphological separation of *S. oppositifolia* ssp. *blepharophylla* from *S. oppositifolia* ssp. *oppositifolia* is impossible over c. 3 km along the main watershed of the Alps because of the predominance of morphological intermediates (P. Schönswetter & A. Tribsch, pers. observ.). Consequently, hybridization might be responsible for haplotype sharing among these taxa. In contrast, despite forming occasional hybrids with *S. oppositifolia* ssp. *oppositifolia* (e.g. Hayek, 1905), *S. oppositifolia* ssp. *rudolphiana* shows no signs of at

least maternal introgression as it harbours an exclusive haplotype (Fig. 1). Recent hybridization can be excluded as a mechanism explaining haplotype sharing between *S. oppositifolia* ssp. *oppositifolia* and *S. retusa* ssp. *retusa*, as these species are not known to hybridize (e.g. Hayek, 1905; P. Schönswetter & A. Tribsch, pers. observ.). Ancient hybridization between these taxa might, however, have given rise to *S. oppositifolia* ssp. *rudolphiana*, which resembles compact forms of *S. oppositifolia* ssp. *oppositifolia* morphologically, but possesses an exclusive haplotype

(h31) derived by a single mutational step from the haplotype (h30) found exclusively in eastern Alpine *S. retusa* ssp. *retusa* (Fig. 1). Ancient plastid capture may also be responsible for the presence of a highly divergent haplotype (h32) in both subspecies of *S. retusa* in the south-western Alps, but this hypothesis needs to be tested in future genus-wide phylogenetic studies.

Despite extensive hybridization and occasional sympatric occurrence in adjacent habitats (A. Tribsch & P. Schönswetter, pers. observ.), the integrity of taxa with respect to nuclear genetic data has not been eroded (Fig. 2). This may be a result of phases of allopatric differentiation in isolated Pleistocene refugia (Schönswetter & Tribsch, 2005) and/or ecological divergence through adaptation to different habitats in terms of elevational range, scree mobility, length of snow cover and bedrock (Grabherr & Mucina, 1993; Hörandl & Gutermann, 1994; Schönswetter, Schneeweiss & Englisch, 2000; Fischer *et al.*, 2008; Table 1). The impact of Pleistocene range shifts on the genetic structure is evident from genetic patterns in *S. retusa* ssp. *retusa*. A close relationship of populations from the Carpathians and the eastern Alps was suggested by strongly weighted splits in the NeighborNet analysis of the AFLP data and the correspondingly high BS (Fig. 2). Accordingly, we presume that *S. retusa* ssp. *retusa* was once more widespread throughout the Alps and survived the most extensive glaciations only in the south-western and eastern-most Alps. In the latter area, almost all contemporary populations are restricted to formerly unglaciated areas outside or close to the margin of the Pleistocene ice sheet (Schneeweiss & Schönswetter, 1999), reflecting the location of Pleistocene refugia. The progenitor/derivative relationship between the Carpathian and the eastern Alpine haplotypes (h28 and h30, respectively), albeit based only on a single nucleotide difference, may indicate that *S. retusa* ssp. *retusa* migrated from the Carpathians into the Alps.

Our study provides compelling evidence that *S. biflora*, *S. oppositifolia* ssp. *blepharophylla*, *S. oppositifolia* ssp. *rudolphiana* and *S. oppositifolia* ssp. *speciosa* merit taxonomic recognition. In contrast with *S. biflora* and *S. retusa* s.l., recognition of which at the species level remains undisputed (Webb & Gornall, 1989; Aeschimann *et al.*, 2004; Fischer *et al.*, 2008), the taxonomic rank of the other taxa is controversial (e.g. Webb & Gornall, 1989 vs. Fischer *et al.*, 2008). Here, we argue that *S. oppositifolia* ssp. *blepharophylla*, *S. oppositifolia* ssp. *rudolphiana* and *S. oppositifolia* ssp. *speciosa* should be recognized at the species level (as *S. blepharophylla* Kern. ex Hayek, *S. rudolphiana* Hornsch. ex W.D.J.Koch and *S. speciosa* Dörf. & Hayek) for several reasons: (1) based on nuclear DNA divergence, *S. blepharophylla*, *S. rudol-*

phiana and *S. speciosa* are similarly differentiated from *S. oppositifolia* as are *S. biflora* and *S. retusa* s.l. (Fig. 2); (2) genetic integrity of these species is retained despite widespread hybridization with *S. oppositifolia*; (3) each species is morphologically and ecologically well characterized (e.g. Grabherr & Mucina, 1993; Table 1). No taxonomic changes are necessary for *S. biflora* (for the reasons outlined in the 'Study species' section, no intraspecific entities need to be recognized) or for *S. retusa* ssp. *retusa* and *S. retusa* ssp. *augustana*, which, because of their moderate nuclear divergence, are best kept as subspecies.

The most complex taxon of purple saxifrages is *S. oppositifolia* ssp. *oppositifolia*, which is also the most widespread member of the genus (Webb & Gornall, 1989). It is morphologically highly variable with several conspicuous forms described. In Europe, this concerns, for example, *S. meridionalis*, characterized by large leaves tapering towards their base (Hayek, 1905). Our samples from the type locality in the Montenegrin Komovi mountain range (population 39-opp), however, exhibit no AFLP divergence from morphologically typical *S. oppositifolia* ssp. *oppositifolia* from the Bulgarian Pirin mountains (population 38-opp). Likewise, the western Alpine *S. murithiana* (populations 41-opp and 42-opp; Hayek, 1905), characterized by glandular sepals, and the morphologically conspicuous Pyrenean *S. oppositifolia* ssp. *paradoxa* (Vargas, 2003), with alternate leaves (populations 60-par and 61-par), are both deeply nested in *S. oppositifolia* ssp. *oppositifolia* (Fig. 2). This strongly suggests that *S. meridionalis*, *S. murithiana* and *S. oppositifolia* ssp. *paradoxa* should not be taxonomically recognized and should be sunk into *S. oppositifolia* ssp. *oppositifolia*. Similar opinions on *S. asiatica* from central Asia and the arctic intraspecific taxa of *S. oppositifolia* were expressed on the basis of morphology by Webb & Gornall (1989) and Aiken *et al.* (2005), respectively, but corroboration from molecular data is still largely lacking. Even if these taxa were retained, *S. oppositifolia* ssp. *oppositifolia* remains a morphologically highly variable, phylogenetically incoherent group (Fig. 2). In the Arctic, an additional level of complexity is introduced by polyploidy, which is involved in local adaptation and growth form (Eidesen *et al.*, 2013). In order to avoid retaining entities that can only be discriminated by geography, we thus suggest treating the genetic clusters as informal phylogeographical groups (similar to the treatment of *Papaver alpinum* L. phylogroups; Schönswetter *et al.*, 2009). In accordance with Hörandl (2006), we do not consider the phylogenetically incoherent state of *S. oppositifolia* as taxonomically problematic, but rather as an unavoidable result of the diversification processes within *Saxifraga* section *Porphyryon* subsection *Oppositifoliae*.

Overall, the predominantly nuclear AFLP data suggest a taxonomic concept in agreement with morphology. Plastid markers, in contrast, show extensive haplotype sharing as a result of either incomplete lineage sorting or hybridization (Maddison, 1997). As a consequence, barcoding with plastid markers (Kress & Erickson, 2007) would lead to fundamentally incorrect phylogenetic conclusions (Fazekas *et al.*, 2009). This is especially true for the strongly divergent haplotype h32 exclusively present in *S. retusa* from the south-western Alps. To place this haplotype in a broader evolutionary context, a new and well-resolved phylogenetic analysis based on multiple samples of all Alpine saxifrages is needed. Despite frequent co-occurrence and hybridization (Gugerli, 1997) and incomplete reproductive isolation, the integrity of taxa is preserved (Soltis & Soltis, 2009), suggesting a crucial role of post-pollination barriers and ecological differentiation of species in terms of adaptation to divergent habitats. Further research should aim at unravelling these isolation mechanisms responsible for the maintenance of the integrity of taxa.

DATA ACCESSIBILITY

Original plastid DNA alignment and AFLP matrix: DRYAD entry: <http://doi.org/10.5061/dryad.8d820>.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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APPENDIX

Table A1. Population numbers (including taxon abbreviation), sampling localities including geographical coordinates, number of individuals analysed, plastid haplotypes, GenBank accession numbers (*psbA-trnH* intergenic spacer; *trnT-F* region), herbarium information and collectors of the 84 populations of *Saxifraga* section *Porphyron* subsection *Oppositifoliae* used in the present study. Populations included in our previous study on *S. oppositifolia* s.s. (Winkler *et al.*, 2012) are marked with an asterisk.

Population*	Locality	Latitude N	Longitude E	N	Plastid haplotype	GenBank accession	Herbarium (voucher)	Collector†
01-opp*	Canada, Newfoundland, Northern Peninsula, Eddis Cove West	50.756	−57.217	1	h3	JX131382; JX131496	O (SUP-3037)	AB, IA
02-opp*	Greenland, Kong Christian X's Land, Myggbukta	72.213	−23.643	1	h9	JX131383; JX131497	O (SUP-3008)	OG
05-opp*	Greenland, Qaanaaq, Agpat Agpai, Little Auk	76.094	−68.326	1	h9	JX131388; JX131502	O (AK-4624)	KW
16-opp*	Russia, Chukotka, Wrangel Island, Somnitelnaya	70.950	−179.417	2	h19	JX131412–JX131413; JX131526–JX131527	O (05/0883)	HS, RE
17-opp*	Russia, Sakha Republic, River Lena: Cherukova	71.048	127.523	2	h22	JX131414–JX131415; JX131528–JX131529	O (SUP-4007)	HS, RE
18-opp*	Russia, Sakha Republic, estuary of River Lena, Sokol	72.402	126.798	2	h19	JX131416–JX131417; JX131530–JX131531	O (SUP-4087)	HS, RE
19-opp*	Russia, Sakha Republic, River Lena: Kharaulakh Mountains	71.925	127.318	2	h19	JX131418–JX131419; JX131532–JX131533	O (SUP-3895)	HS, RE
21-opp*	Russia, Taimyr, Anabar Plateau, Kayak (upper valley of river Kotuy)	71.505	103.057	2	h19	JX131422–JX131423; JX131536–JX131537	WU (T510)	ATR, PS
23-opp*	Russia, Franz Joseph Land, Cape Flora	79.950	50.083	1	h1	JX131425; JX131539	DNA-SB Oslo	MC
25-opp*	Russia, Novaya Zemlya, Guba Gribovaya	73.008	53.236	2	h1	JX131428–JX131429; JX131542–JX131543	DNA-SB Oslo	HST
26-opp*	Russia, Komi ASSR, Yugyd-Va National Park	65.341	60.712	2	h1, h2	JX131430–JX131431; JX131544–JX131545	O (SUP-4448)	ATR, IA
27-opp*	Norway, Finnmark, Batsfjord	70.643	29.712	1	h9	JX131432; JX131546	O (SUP-4555)	KH, OS
29-opp*	Norway, Oppland, Vang/ Vågå, Jotunheimen, Torfinnsdalen	61.383	8.583	1	h7	JX131434; JX131548	WU (N12)	ATR, PS
30-opp*	Russia, Altai Republic, Altai, Aktash	50.331	87.736	2	h23, h25	JX131435–JX131436; JX131549–JX131550	WU (9498)	ATR
31-opp*	Russia, Altai Republic, Juzhno Chujskij Khrebet, River Zhasater	49.658	88.175	2	h23	JX131437–JX131438; JX131551–JX131552	WU (9579)	ATR
32-opp*	Russia, Altai Republic, Altai, Akkol, Sofijskij glacier	49.814	87.808	1	h26	JX131440; JX131554	WU (9614)	ATR
33-opp*	Kirgistan, Central Tien Shan, Tjuz pass	42.183	79.667	2	h18	JX131441–JX131442; JX131555–JX131556	Z (14.xiii.2005)	FG
34-opp*	Kirgistan, Central Tien Shan, Engylchek glacier	42.175	79.712	1	h18	JX131443; JX131557	Z (15.xiii.2005)	FG
35-opp*	Slowakia, Belanské Tatry, Tristarska Dolina	49.263	20.204	2	h5, h9	JX131444–JX131445; JX131558–JX131559	WU (10593)	MR, PS
37-opp*	Romania, Munții Bucegi, Cabana Babele	45.415	25.471	1	h28	JX131448; JX131562	WU (10505)	OP, PS
38-opp*	Bulgaria, Pirin, Vihren	41.761	23.405	2	h29	JX131450–JX131451; JX131564–JX131565	WU (11337)	BFR, PS
39-opp*	Montenegro, Komovi, Kom Kučki	42.681	19.642	2	h28	JX131452–JX131453; JX131566–JX131567	WU (11749)	BFR, PS
40-spe*	Italy, L'Aquila, Abruzzo, Gran Sasso, Monte Camicia	42.430	13.700	2	h27	JX131454–JX131455; JX131568–JX131569	WU (8924)	GMS, PS
41-opp*	Italy, Piemonte, Alpi Ligurie, Monte Rotondo	44.203	7.785	1	h8	JX131456; JX131570	WU (5305)	ATR, GMS, PS

Table A1. *Continued*

Population*	Locality	Latitude N	Longitude E	N	Plastid haplotype	GenBank accession	Herbarium (voucher)	Collector†
42-opp*	Italy, Aosta, Alpi Graie, Valle di Champorcher, Cima Beccher	45.618	7.557	2	h1	JX131457–JX131458; JX131571–JX131572	WU (5348)	ATR, GMS, PS
42-aug	Italy, Aosta, Alpi Graie, Valle di Champorcher, Cima Beccher	45.618	7.557	1	h32	KF270790; KF270855	WU (4796)	ATR, PS
43-bif	Switzerland, Valais, Dent de Morcles	46.206	7.071	1	h1	KF270748; KF270813	WU (9312)	MW
43-opp*	Switzerland, Valais, Dent de Morcles	46.206	7.071	2	h1	JX131459–JX131460; JX131573–JX131574	WU (9313)	MW
44-bif	Switzerland, Bern, Gemmi, Rot Totzig, Tälligletscher	46.422	7.593	2	h10	KF270749–KF270750; KF270814–KF270815	Z (447)	RH
44-opp*	Switzerland, Bern, Gemmi, Rot Totzig, Tälligletscher	46.411	7.596	2	h1	JX131461–JX131462; JX131575–JX131576	Z (446)	RH
45-bif	Switzerland, Bern, Adelboden, Tschingellochtihorn	46.444	7.594	2	h1	KF270751–KF270752; KF270816–KF270817	WU (9388)	SE
45-opp*	Switzerland, Bern, Adelboden, Tschingellochtihorn	46.444	7.594	2	h1	JX131463–JX131464; JX131577–JX131578	WU (9387)	SE
46-bif	Switzerland, Wallis, Simplon, Kaltwassergletscher, Chaltwassertäli	46.258	8.074	1	h12	KF270753; KF270818	Z (444)	RH
46-opp*	Switzerland, Wallis, Simplon, Kaltwassergletscher	46.260	8.079	2	h1	JX131465–JX131466; JX131579–JX131580	Z (445)	RH
47-opp*	Switzerland, Ticino, Bosco Gurin, Krameggpass	46.316	8.442	1	h9	JX131468; JX131582	Z (439)	RH
47-ret	Switzerland, Ticino, Bosco Gurin, Krameggpass	46.316	8.442	1	h1	KF270794; KF270859	Z (438)	RH
48-bif	Switzerland, Glarus, Braunwald, Ortstock, Furgelle	46.930	8.941	2	h1, h12	KF270754–KF270755; KF270819–KF270820	Z (436)	RH
48-opp*	Switzerland, Glarus, Braunwald, Ortstock	46.925	8.948	2	h1, h13	JX131469–JX131470; JX131583–JX131584	Z (437)	RH
49-bif	Switzerland, Graubünden, Mulegns, Val Bercla, Fuorcla da Faller	46.474	9.500	1	h1	KF270756; KF270821	Z (441)	FG, RH
49-opp*	Switzerland, Graubünden, Mulegns, Val Bercla, Fuorcla da Faller	46.474	9.584	1	h12	JX131472; JX131586	Z (440)	FG, RH
50-opp*	Switzerland, Graubünden, Albula, Fuorcla Valetta	46.534	9.816	2	h1	JX131473–JX131474; JX131587–JX131588	WU (9255)	MW, PS
51-opp*	Italy, Südtirol, Dolomiten, Fanes, Piz de Lavarela	46.585	11.971	2	h1, h10	JX131475–JX131476; JX131589–JX131590	WU (9480)	AH
52-opp*	Italy, Südtirol, Dolomiten, Seekofel	46.669	12.081	2	h12, h14	JX131477–JX131478; JX131591–JX131592	WU (9479)	AH
53-bif	Austria, Osttirol, Hohe Tauern, Lasörllinggruppe, Dabertal	46.997	12.240	2	h12	KF270757–KF270758; KF270822–KF270823	WU (8980)	PS
53-opp*	Austria, Osttirol, Hohe Tauern, Lasörllinggruppe, Dabertal	46.997	12.240	2	h10, h12	JX131479–JX131480; JX131593–JX131594	WU (8982)	PS
53-rud	Austria, Osttirol, Hohe Tauern, Lasörllinggruppe, Dabertal	46.985	12.240	2	h31	KF270775–KF270776; KF270840–KF270841	WU (8981)	PS
54-opp*	Italy, Belluno, Prealpi Carniche, Monte Tiarfin	46.463	12.587	2	h1	JX131481–JX131482; JX131595–JX131596	WU (8957)	GMS, PS

Table A1. Continued

Population*	Locality	Latitude N	Longitude E	N	Plastid haplotype	GenBank accession	Herbarium (voucher)	Collector†
55-bif	Austria, Salzburg, Hohe Tauern, Glockner-Gruppe, Grosses Wiesbachhorn	47.169	12.736	1	h9	KF270759; KF270824	WU (8987)	PS
55-opp*	Austria, Salzburg, Hohe Tauern, Glockner-Gruppe, Grosses Wiesbachhorn	47.169	12.736	2	h1	JX131483–JX131484; JX131597–JX131598	WU (8986)	PS
55-rud	Austria, Salzburg, Hohe Tauern, Glockner-Gruppe, Grosses Wiesbachhorn	47.169	12.736	2	h31	KF270777–KF270778; KF270842–KF270843	WU (8987)	PS
56-opp*	Austria, Kärnten/ Salzburg, Hohe Tauern, Goldberggruppe, Schareck	47.069	12.860	1	h4	JX131485; JX131599	WU (070731)	CT, FG
56-rud	Austria, Kärnten/ Salzburg, Hohe Tauern, Goldberggruppe, Schareck	47.069	12.860	3	h31	KF270779–KF270781; KF270844–KF270846	WU (070730)	CT, FG
57-opp*	Austria, Salzburg, Niedere Tauern, Radstädter Tauern, Rothenkarscharte	47.194	13.472	2	h1	JX131486–JX131487; JX131600–JX131601	WU (8965)	PS
57-rud	Austria, Salzburg, Niedere Tauern, Radstädter Tauern, Rothenkarscharte	47.194	13.472	1	h31	KF270782; KF270847	WU (8966)	PS
58-ble	Austria, Steiermark, Niedere Tauern, Wölzer Tauern, Rettlkirchspitze	47.263	14.133	2	h1, h11	KF270762–KF270763; KF270827–KF270828	WU (9184)	CS
58-opp*	Austria, Steiermark, Niedere Tauern, Wölzer Tauern, Rettlkirchspitze	47.263	14.133	1	h1	JX131488; JX131602	WU (9182)	CS
58-rud	Austria, Steiermark, Niedere Tauern, Wölzer Tauern, Rettlkirchspitze	47.263	14.133	2	h31	KF270783–KF270784; KF270848–KF270849	WU (9179)	CS
59-ble	Austria, Steiermark, Niedere Tauern, Wölzer Tauern, Hoher Zinken	47.271	14.342	1	h12	KF270764; KF270829	WU (9072)	ATR
59-opp*	Austria, Steiermark, Niedere Tauern, Wölzer Tauern, Hoher Zinken	47.271	14.342	2	h1, h12	JX131489–JX131490; JX131603–JX131604	WU (9071)	ATR
59-ret	Austria, Steiermark, Niedere Tauern, Wölzer Tauern, Hoher Zinken	47.271	14.342	2	h30	KF270795–KF270796; KF270860–KF270861	WU (9173)	ATR
60-par*	Spain, Catalunya, Girona, Puigmal d'Err	42.365	2.148	2	h1	JX131491–JX131492; JX131605–JX131606	WU (8825)	GMS, PS
61-par*	Spain, Aragón, Huesca, Sierra Marqués, Collado de Cao	42.650	0.267	2	h1, h16	JX131493–JX131494; JX131607–JX131608	WU (8861)	GMS, PS
62-opp*	Spain, Cantabria, Picos de Europa, Collada del Medio	43.317	–3.300	1	h15	JX131495; JX131609	WU (8895)	GMS, PS
63-aug	France, Alpes Maritimes, Col de la Lombarde, Passo d'Orgials	44.203	7.149	1	h32	KF270791; KF270856	WU (4692)	ATR, PS
64-aug	Italy, Piemonte, Alpi Cozie, Monviso, Colle del Viso	44.668	7.105	1	h32	KF270792; KF270857	WU (6359)	ATR, GMS, PS
65-ret	Italy, Piemonte, Alpi Cozie, Punta Cialancia	44.876	7.122	2	h32	KF270797–KF270798; KF270862–KF270863	WU (4735)	ATR, PS
66-aug	Italy, Piemonte, Alpi Graie, Monte Palon	45.208	7.144	1	h32	KF270793; KF270858	WU (5123)	ATR, PS

Table A1. *Continued*

Population*	Locality	Latitude N	Longitude E	N	Plastid haplotype	GenBank accession	Herbarium (voucher)	Collector†
67-ret	Italy, Aosta, Alpi Pennine, Val d'Ayas, Monte Nery	45.765	7.743	1	h17	KF270799; KF270864	WU (070732)	ATR, GMS, PS
68-rud	Italy, Südtirol, Hohe Tauern, Venedigergruppe, Rötspitze	47.036	12.196	2	h31	KF270785–KF270786; KF270850–KF270851	No voucher	CT
69-bif	Austria, Kärnten, Hohe Tauern, Ankogelgruppe, Grauleitenspitze	47.046	13.218	2	h1, h6	KF270760–KF270761; KF270825–KF270826	WU (9082)	ATR
69-ble	Austria, Kärnten, Hohe Tauern, Ankogelgruppe, Grauleitenspitze	47.046	13.218	3	h1 (1), h9 (2)	KF270765–KF270767; KF270830–KF270832	WU (9084)	ATR
69-rud	Austria, Kärnten, Hohe Tauern, Ankogelgruppe, Grauleitenspitze	47.046	13.218	1	h31	KF270787; KF270852	WU (9083)	ATR
70-ble	Austria, Kärnten, Hohe Tauern, Hafner, Marschneid	47.071	13.400	1	h10	KF270768; KF270833	WU (9186)	ATR, PS
71-ble	Austria, Kärnten, Hohe Tauern, Hafnergruppe, Elendkar	47.018	13.500	2	h12	KF270769–KF270770; KF270834–KF270835	WU (8970)	PS
71-rud	Austria, Kärnten, Hohe Tauern, Hafnergruppe, Elendkar	47.018	13.500	2	h31	KF270788–KF270789; KF270853–KF270854	WU (8971)	PS
72-ble	Austria, Steiermark, Niedere Tauern, Schladminger Tauern, Hochwildstelle	47.336	13.829	2	h1, h11	KF270771–KF270772; KF270836–KF270837	WU (9187)	ATR, PS
73-ble	Austria, Steiermark, Niedere Tauern, Schladminger Tauern, Predigtstuhl	47.260	13.910	2	h9, h10	KF270773–KF270774; KF270838–KF270839	WU (9188)	ATR, PS
74-ret	Austria, Steiermark, Niedere Tauern, Wölzer Tauern, Hornfeldspitze	47.267	14.092	2	h30	KF270800–KF270801; KF270865–KF270866	WU (9194)	ATR, PS
75-ret	Austria, Steiermark, Niedere Tauern, Wölzer Tauern, Straßbeck	47.256	14.140	2	h30	KF270802–KF270803; KF270867–KF270868	WU (070729)	ATR, PS
76-ret	Austria, Steiermark, Seetaler Alpen, Zirbitzkogel	47.067	14.567	2	h30	KF270804–KF270805; KF270869–KF270870	WU (9192)	ATR, PS
77-ret	Poland, Tatry Wysokie, Polski Grzebień, Litworowy Staw	49.174	20.140	1	h28	KF270806; KF270871	WU (10606)	MR, PS
78-ret	Romania, Bistrița, Munții Rodnei, Ineuł	47.526	24.884	2	h28	KF270807–KF270808; KF270872–KF270873	WU (070728)	AR, MR
79-ret	Romania, Argeș, Munții Făgărașului, Arpașu	45.595	24.656	2	h28	KF270809–KF270810; KF270874–KF270875	WU (070727)	AR, MR
80-ret	Romania, Argeș, Munții Făgărașului, Tarata	45.606	24.695	2	h28	KF270811–KF270812; KF270876–KF270877	WU (070726)	AR, MR

*aug, *S. retusa* ssp. *augustana*; bif, *S. biflora* ssp. *biflora*; ble, *S. oppositifolia* ssp. *blepharophylla*; opp, *S. oppositifolia* ssp. *oppositifolia*; par, *S. oppositifolia* ssp. *paradoxa*; rud, *S. oppositifolia* ssp. *rudolphiana*; ret, *S. retusa* ssp. *retusa*; spe, *S. oppositifolia* ssp. *speciosa*.

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